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REVIEW

Passage to the Terrestrial Life in Amphibians: Events Accompanying This Ecological Transition

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CONTENTS

ABSTRACT—Passing from an aquatic environment to a terrestrial one has been analyzed in developing amphibians. The emergence occurs at metamorphosis in many species, while appear dramatic changes of the organizational plan. Metamorphosed animals acquire another type of locomotion and respiration. Striking transformations take place in the digestive tract and result in modifications of the feeding behaviour. Water conservation problem is solved by appearance of a new hydromineral equilibrium, which is associated to another type of nitrogen excretion. Nevertheless, such events do not completely free juveniles and adults from an aquatic environment. Other amphibians acquire a direct development which puts an end to the aquatic larval stage. This more radical departure from water exists in several oviparous species and also in various other species in which the offspring-parental relationship is more or less pronounced.

INTRODUCTION

Water is the source of life, and passing from an aquatic environment to a terrestrial one is always a major problem. This ecological transition is particularly obvious in developing amphibians. The present article analyzes the departure from an aquatic environment during metamorphosis. The

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associated changes have been studied not only at the anatomo-morphological level, but also at the physiological and biochemical level [4, 21, 23, 33, 42, 56, 89, 90, 96].

A more radical departure will also be discussed for certain amphibians in which the aquatic larval stage disappears. The endocrine determinism presiding this ecological transition will be analyzed in another article (in press).

I A SERIES OF METAMORPHIC CHANGES ACCOMPANIES THE DEPARTURE FROM AN AQUATIC ENVIRONMENT IN AMPHIBIANS

A ACQUIRING ANOTHER TYPE OF-LOCOMOTION

1 *The tail, swimming organ in premetamorphic aquatic larvae*

The tail of amphibian larvae is made up of a fleshy axis with a membranous dorso-ventral fin. The rythmic undulations of the tail give it an essentially propulsive function. Swimming can be very rapid when the tail is well developed with powerful muscles as in *Astylosternus* and *Leptodactylodon*. Caudal undulations are controlled by the activity of two giant neurons (Mauthner neurons), in which the axons follow the same path as the spinal cord [69]. Such neurons enhance the immediate flight reflex after a mechanical or sound stimulation (impact against the side of the aquarium). The nerve impulse is then transmitted to the Mauthner neurons by the stato-acoustic nerves.

The degree of water-oxygenation conditions larval endurance. Tadpoles in a well oxygenated environment receive enough chemical energy furnished by the ATP from respiratory catabolism and consequently tire very little. The chemical energy in poorly oxygenated water is lower since it comes from anaerobic catabolism (lactin fermentation), and the animals become incapable of furnishing a highly endoenergetic sustained propulsion effort [86].

2 *Regression of the tail during anuran metamorphosis*

The degeneration of the tail and its complete regression characterizes the climax stage (paroxysmal stage) of metamorphosis. To the naked eye, the first signs of degeneration consist in a thinning of the fin and a darkening of the end of the tail due to an accumulation of melanophores. All the tail tissues are altered [29, 88]. The spinal cord, notochord and epidermis are autolyzed: the cell constituents are segregated in the lysosomes (autophagic vacuoles) where they are digested by hydrolases (cathepsins). The muscle fibers retract and become individualized, thus deforming the tail. The myofibrils break down and the actin and myosin myofilaments become disorganized (Fig. 1). These processes result in the appearance of

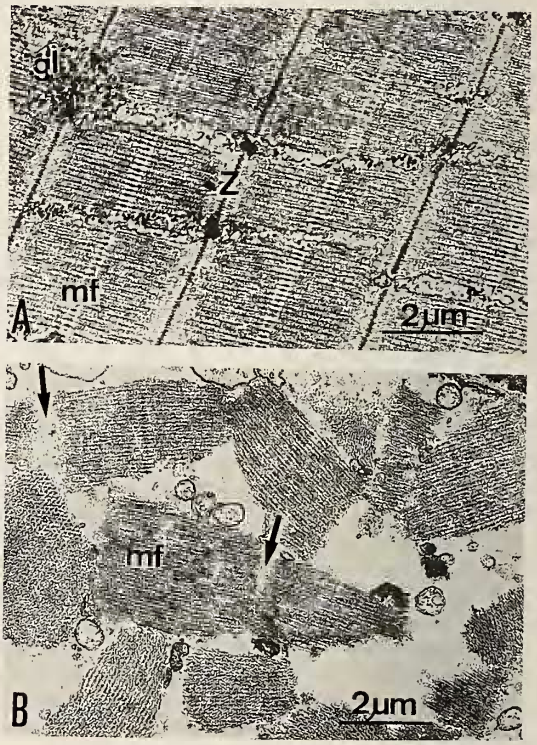

Fig. 1. Electron micrograph of a longitudinal section of a caudal striated muscle fiber from an *Alytes obstetricans* tadpole. *A*, Premetamorphosis. *B*, Climax. The Z bands (Z) are disrupted during climax (arrows) and produce a fragmentation of the myofibrills (mf). gl, glycogen. Unpublished micrography by Pouyet.

bundles of fragmented myofibrils enclosed in round bodies. Enzymes such as actinase and myosinase could be responsible for these alterations [12, 49, 97].

The existence of phagocytic processes was demonstrated in the first studies concerning caudal regression. The caudal fibroblasts undergo a metabolic reorientation and become capable of absorbing and totally digesting the cell fragments thanks to the lysosomal hydrolases contained in their digestive vacuoles [30, 48, 91]. The fibroblasts also secrete hyaluronidase, which they use to partially liquify the connective tissue, thereby coming closer to the alteration sites.

3 *Locomotion in metamorphosed anurans*

a *Adaptations to jumping.* This is due to the spectacular growth of the hindlimbs. External buds until metamorphosis, the limbs rapidly lengthen at the onset of the phenomenon, preliminary to acquiring a jumping type of locomotion in terrestrial habitat. This adaptation benefits from a distortion of the pelvic girdle which places the cotyloid cavity, articulated with the femoral head, in a very posterior position. A stocky body with a relatively short spinal column also makes jumping easier. The forelimbs are used when landing on the ground. They develop in the gill cavities and emerge during climax. When the animals return to a pond to reproduce or to escape from a predator, the springing action of the hindlimbs combined with palmed toes results in a strong swimming capacity.

b *Skelettal ossification.* During metamorphosis, skelettal remodeling is associated with a significant ossification which implies a modification of the calcium metabolism and phosphate and calcium carbonate deposits in the skeleton.

Skeletal calcium comes from the tadpoles two endolymphatic sacs and the chalky sacs. Each endolymphatic sac comes from the endolymphatic canal which develops at the internal ear level. These sacs slip between the skull and the brain into the spinal cord canal during metamorphosis. The endolymphatic and chalky sacs contain minute calcium concretions (aragonite). These deposits are largely resorbed during metamorphosis and the calcium thus mobilized contributes to skeletal ossification [65].

The skeletal ossification of the tadpole is enhanced by the thyroid hormones, as observed during induced metamorphosis [47]. Calcium metabolism is also controlled by the ultimobranchial bodies [15, 68]. These bodies favor calcium storage in the endolymphatic and chalky sacs of the tadpole, a prerequisite for proper ossification during metamorphosis.

c *Effect of nervous system maturation on locomotion in a terrestrial environment.* The new locomotor behaviour acquired by metamorphosed anurans is supported by a mature cerebellum. The development of the dendrites and axons of the granular and Purkinje cells in this organ and the granular cells migration results in a large number of synapses [39, 82]. This conditions the appearance of coordinated jumping movements. Furthermore, the cerebellum controls the muscular tonicity which counterbalances the effect of gravity in a terrestrial environment.

Dendrite endings curled around muscle fibers (neuro-muscular spindles) have been identified in the hindlimbs of *Rana pipiens* during metamorphosis [10]. Such endings are sensitive to the degree of fiber contraction and make it easier to control the jump.

4 *The case of urodeles*

The urodeles tail does not undergo spectacular morphological modifications during metamorphosis, since only the fin regresses. The limbs lengthen moderately. The juveniles locomotion on land, however, is limited to simple reptation movements by the limbs transversal disposition.

B RESPIRATORY CHANGES

Amphibian larvae and adults have a wide range of respiratory exchangers, which allow a very progressive transition without acute respiratory failure during metamorphosis and the passage to a terrestrial environment [3, 42].

1 *Respiratory system in larvae*

Arborescent gills are present during the larval stage. Each gill consists of a stem from which rise gill filaments. These filaments are covered with a simple, flat epithelium which is practically next to the blood vessels. The skin, with its very dense capillary network, furnishes a second major respiratory exchanger. Moreover, many amphibian larvae have lungs, which probably have an early

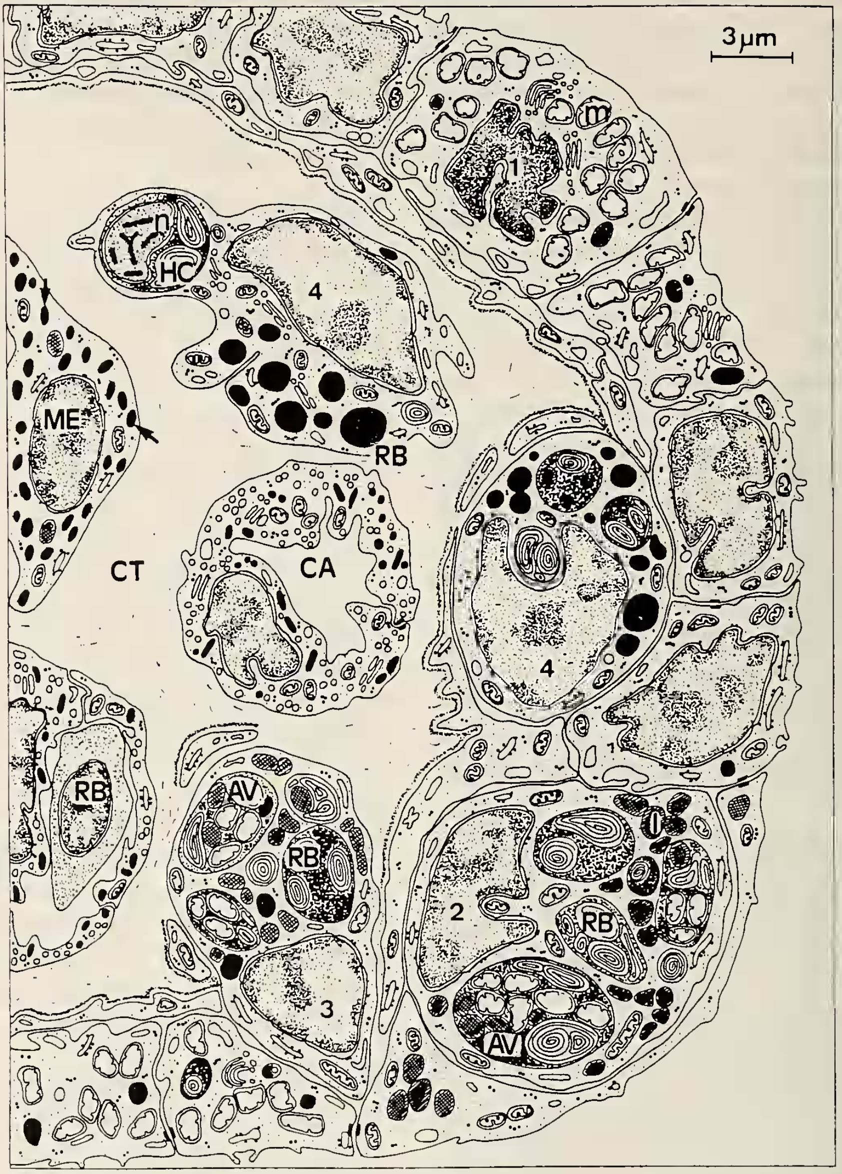

Fig. 2. Metamorphic *Discoglossus pictus* tadpole: ultrastructural features of the degenerating gills (level of the stem). Some epitheliocytes (1) undergo a diffuse autolysis and their mitochondria are dilated (m). Other epitheliocytes (2) contain autophagic vacuoles (AV), residual bodies (RB) and lipid droplets (l). These cells might be extruded (3) in the connective tissue (CT). Phagocytic cells (4) are also seen. They contain heterophagic vacuoles (HC) and residual bodies (RB). The capillaries (CA) have a shrunken lumen. On the left, a melanophore (ME) can be recognized by its very dark pigment granules (arrows). RB, red blood cell; m, mitochondrion; n, nuclear remnant. From Hourdry [41].

but very accessory function(1) [9].

When the water is poor in oxygen (hypoxic conditions), the larvae hyperventilate with their lungs by frequently swallowing air at the surface [24, 94]. After each intake, the flow of water in the gills decreases temporarily. This is due to a stimulation of the mechanoreceptors sensitive to oxygen pressure in the lung wall and the chemoreceptors sensitive to oxygen concentration in the blood. When hypoxic conditions persist, the respiratory exchanges of the larvae undergo extensive alterations [8]: the blood vessels multiply and become more superficial; the number of gill filaments increases. These observations underline the highly plastic morphology of these exchangers.

2 *Changes in the respiratory system during metamorphosis*

The gills regress during metamorphosis (Fig. 2) [41]. The epithelium undergoes a diffuse autolysis. Its constituents can also be digested inside the lysosomes (autophagic vacuoles). The altered cells are usually absorbed by mesenchymal cells with phagocytic properties, and are digested inside large lysosomes (heterophagic vacuoles). Gill circulation finally ceases while the gill filaments wither and become pigmented before disappearing.

In most amphibians, septation of the pulmonary cavity follows metamorphosis and continues to progress until the adult stage, thus increasing the lungs internal surface at least fivefold.

Oxygen absorption in metamorphosing anurans takes place essentially through the skin. As soon as the juvenile stage, however, the septated pulmonary epithelium increases its contribution while the skin thickens and partially loses its oxygen absorbing capacity. Nevertheless, the skin remains the primary carbon dioxide excreting organ during and after metamorphosis.

Gill degeneration is associated with extensive changes in the circulatory system. The afferent and efferent gill arteries regress. Aortic arches differentiate and irrigate the head (carotide arch), the trunk and limbs (systemic arch merging and forming the dorsal aorta), the lungs and skin (pulmo-cutaneous trunk).

3 *Changes in oxygen transfers: appearance of new hemoglobins and new red cells*

The larval hemoglobins (Hb. l) in most anurans are different from those that replace them in adults (Hb. ad). In various frogs (*Rana esculenta, Rana catesbeiana*), this substitution accelerates during metamorphosis and continues several weeks after. In the tree-frog (*Hyla arborea*), leopard-frog (*Rana pipiens*) and common toad (*Bufo bufo*), the

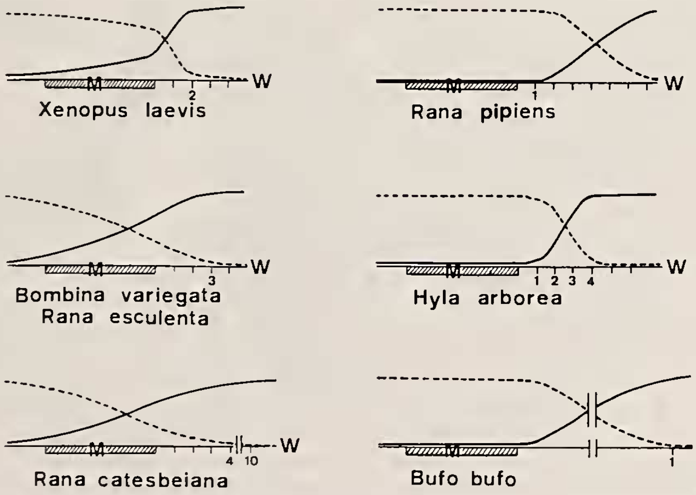

FIG. 3. Shift from larval hemoglobins (dotted lines) to adult hemoglobins (continuous lines) in anurans. M, metamorphosis; w, week; y, year. From Hourdry and Beaumont [42].

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(1) They are absent in bufonidae larvae.

substitution takes place after metamorphosis. (Fig. 3). In urodeles, it can occur slightly before (*Pleurodeles waltl, Triturus cristatus*) or during (*Ambystoma tigrinum*) metamorphosis.

The change in hemoglobins has been demonstrated by various analytical techniques. Electrophoresis, for example, produces different bands in anurans [46, 60, 66] as well as in urodeles [27]. The differences found between Hb. l and Hb. ad are only located on one globin and never affect the prosthetic group (tetraheme). The absence of common polypeptide chains has been irrefutably proven by the presence of different amino acid sequences in the different hemoglobin families.

During hemoglobin substitution, the iron from the Hb. l accumulates as ferritin in the Kupffer cells of the liver. The metal will eventually be carried by the plasma transferrins and incorporated into the new hemoglobins at their site of synthesis (essentially bone marrow) [7, 63, 80, 83].

Hb. l and Hb. ad have different affinities for oxygen [78]. The Hb. l in premetamorphic anurans have a high oxygen affinity. This is compatible with an aquatic environment where the concentration of dissolved oxygen is particularly low. Hb. ad have a lower oxygen affinity. Hb. ad production is associated with the presence of new and more numerous red cells, developed in different sites (liver, bone marrow, *etc.*), and which replace those of the larva [92, 93].

C CHANGES IN THE DIGESTIVE TRACT. EFFECT ON THE FEEDING BEHAVIOUR

During metamorphic climax, the digestive tract is modified, especially in anurans. Such changes are associated with modications in the feeding behaviour.

1 *Changes in the digestive tract of anurans*

The size of the buccal cavity increases considerably following the lengthening of the jaws. The horny tadpoles "teeth" disappear while true teeth develop.

In larvae, the pharyngeal gill clefts are covered by a rough epithelium with ridges that form gill filters. These filters strain the water which irrigates the gills and retain the food particles in suspension. During metamorphosis, the pharynx undergoes a substantial reduction. It no longer plays a part in respiration since the gills and gill clefts regress. The gill filters also degenerate.

During the climax, a dilated stomach is formed. Its epithelium evaginates into the chorion and becomes tubular gastric glands which lengthen and ramify (Fig. 4). The muscle layer thickens. These structural modifications are associated with func-

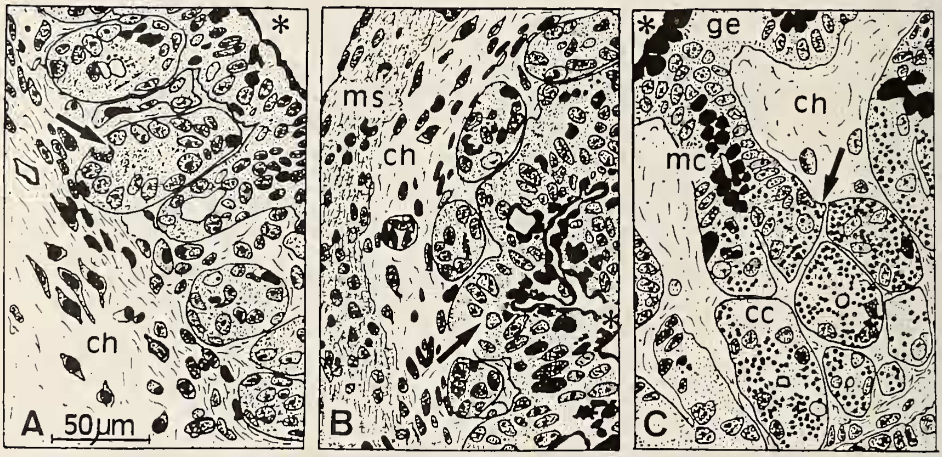

Fig. 4. Histological changes in the gastric region during *Alytes obstetricans* metamorphosis. *A* and *B*, Onset of climax. The epithelium evaginates into the chorion (ch) and becomes gastric glands (arrows). Asterisk, lumen; ms, muscle layer. *C*, End of climax. The gastric glands are completely differentiated (arrow): they each have a neck of mucous cells (mc) into which open tubules made up of granulated serous cells (cc or chief cells). A gastric mucous epithelium (ge) runs along the lumen (asterisk). From unpublished micrographies by Hourdry and Pouyet.

tional changes such as pepsin and hydrochloric acid production, by chief (or pepsinogen) cells [5, 38, 43, 51, 58] and parietal (or oxyntic) cells [28], respectively. Two pepsinogens have been found in the gastric mucosa of the adult bullfrog [85, 95]. These pepsinogens are immunologically indistinguishable from each other and are related to human pepsinogen C. Chitinases are also secreted by the stomach as soon as it differentiates [64].

The metamorphosing intestine undergoes particularly spectacular changes. An overall shortening and rearrangement of its coils occurs concomitantly with the development of the stomach. The histological changes are largely confined to the epithelium [26, 40, 42, 44]. Degeneration occurs in the larval (or primary) epithelium, while the extensive proliferation of stem cells results in the development of the new, folded epithelium (secondary epithelium).

2 *Changes in the type of feeding during anuran development*

a *Premetamorphic larvae.* Most tadpoles are microphagous and feed on particles suspended in the water (bacteria, protozoa, unicellular algae...). Benthic tadpoles, such as those of *Rana temporaria* or *Bufo vulgaris* are herbivorous or detritivorous. Their keratinized "teeth" tear apart the plant organs and scrape the crusts. This results in a suspension of particles which are then swallowed by the animal. The periodic variations of buccal cavity volume, due to the pulsating movements of its wall, create a water intake and result in the ingestion of food particles.

The particles suspended in the water are usually gathered up by retention on the surface of the various buccopharyngeal structures (Fig. 5) [35]. Certain particles are retained by the buccal cavity papillae and by the dorsal and ventral velums. Such particles adhere to the mucous traps that have differentiated in the roof of the buccal cavity. In a few cases, traps can be observed on the underside of the dorsal velum. The other particles are retained by gill filters. After being swept up by water currents, they adhere to the mucous of traps located on the underside of the ventral velum (branchial food traps) [42, 87]. The gill filters and branchial food traps found on the ventral velum make up the tadpole's fltering apparatus. The various mucous secretions are canalized in pharyngeal grooves where they form cords which are conveyed by the ciliary movements toward the

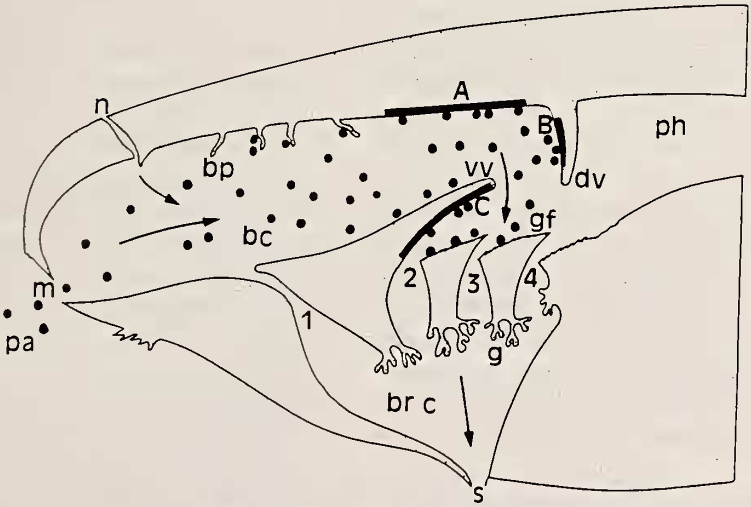

FIG. 5. Parasagittal section of the buccal (bc), pharyngeal (ph) and branchial (br c) cavities in a premetamorphic anuran tadpole. Food particles suspended in the water (pa) are retained by the buccal cavity papillae (bp), dorsal (dv) and ventral (vv) velums, and gill filters (gf). They then adhere to the mucous of traps that have differentiated in the roof of the buccal cavity (A), and on the underside of the dorsal velum (B) and ventral velum (C: branchial food traps). The water flux is shown by the arrows. g, gill; m, mouth; n, nostril; s, spiraculum; 1–4, gill clefts. From Gradwell [36].

oesophagus.

In short, many anuran species have tadpoles which are highly specialized suspension users. Particle retention on the gill filters allows effortless feeding which is compatible with accelerated growth, when trophic conditions are favorable. It is noteworthy that many anuran species lay their eggs after heavy rains which leach the soil and provoke an influx of mineral salts into the ponds, thus stimulating the plankton production on which tadpoles feed.

Cellulose is probably not used by the larvae: the thin muscle layer has a weak peristaltism which is not very efficient for breaking down plant cell walls. Furthermore, the larvae do not have cellulase. Amylase from the pharyngeal grooves and most of all, exocrine portion of the pancreas, has been observed [55]. This suggests a preferential digestion of the starch found in the food particles. Three intestinal brush border glucidases are also implicated in the hydrolysis of dietary glucides (glucoamylase, maltase, trehalase).

Dietary glucides are the source of lipids and hepatic glycogen, whose quantities increase during premetamorphosis and which are the main energy storage sources for the tadpole.

b *Metamorphic and post-metamorphic animals.* Anuran tadpoles usually stop feeding at climax. Indeed, the digestive tract is too altered to function.

In post-metamorphic anurans, the mouth widens and stronger jaws with true teeth appear. An eventually prehensile tongue develops. These various transformations are concurrent with a change in the diet, since the animals become carnivorous of insectivorous, and catch preys with their prehensile tongue. At the same time, the eyes(1) migrate toward the plan of symmetry. Such a migration allows binocular and stereoscopic vision, keen sight and an efficient perception of the preys in a terrestrial environment. Simultaneously, new neuronal connections appear between the retina and the optic tectum [32, 37]. In premetamorphic tadpoles, the tectal electric registrations are solely contralateral. On the other hand, these registrations may be both contralateral and ipsilateral in metamorphosed animals, due to the development of a system or intertectal connections. Each point of the binocular visual space corresponds then to two tectal points through each eye.

The propulsion of solid food is enhanced by the appearance of stronger peristaltic movements due to a thicker muscle layer.

The secretion of proteinases in the pancreas (trypsin) [73] and the stomach (pepsin) prepares the animals for a carnivorous diet. Stomacal chitinases also prepare the metamorphosed animals for an insectivorous diet. Furthermore, the new intestinal mucosa is well equipped for the absorption of protein hydrolysis residues: an active γ-glutamyl transferase acts as a membrane-localized transporter of amino acids and possibly dipeptides [31, 59]. Moreover, this enzyme hydrolyses the γ-glutamic acid-peptide bonds found in physiologically important peptides, such as glutathione and folic acid.

In short, a new feeding behaviour is prepared during anuran metamorphosis by important changes in the digestive tract, while the animals pass from an aquatic to a terrestrial environment.

3 *The case of urodeles*

The metamorphic changes in the urodelean digestive tract are very restricted [42], although many larvae leave the ponds and migrate toward a terrestrial environment. The diet is slightly modified, since the animals are still carnivorous predators.

D TRANSITION TO A NEW HYDROMINERAL EQUILIBRIUM

Amphibiians are confronted with a major water conservation problem in their transition to a terrestrial environment. This problem is solved by a series of adaptations which appear during metamorphosis.

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(1) During metamorphosis, the optical properties of the eye are modified [75, 76]. The tadpole eye is a "fish eye": its spherical lens provides nearly all the ocular convergence. The eye of the metamorphosed anuran has a lenticular lens, and its convergence is mainly due to the cornea, whose refractive index (n=1, 38) is higher than that of air (n=1).

1 *Changes in the serum proteins*

a *Qualitative changes.* These are extensive in metamorphosing anurans [11, 67, 81]. The number of molecular species increases at climax and the band characterizing albumin on an electrophoregram becomes particularly distinct (Fig. 6). These changes are usually more attenuated in urodeles.

FIG. 6. Electrophoretic patterns of serum proteins in metamorphosing larvae of *Rana temporaria* (from left to right). The albumin band (al) has a distinctly higher anodal mobility and becomes thicker and thicker. From Chen [11].

b *Variations in the concentration of serum proteins.* This concentration at least doubles in metamorphosing anurans; in *Rana catesbeiana* for example, it goes from 1.2 g/100 ml to 3 g/100 ml. The increase in serumalbumin is particularly spectacular in this species, from 0.08 g/100 ml to 0.8 g/100 ml, a tenfold increase [25, 54]. Urodeles have weaker variations.

These increases imply a stimulation of liver proteosynthesis. In *Rana catesbeiana*, radioactive precursors have shown that the rate of serumalbumin synthesis is multiplied by 6 at climax [54]. During metamorphosis, the higher serum protein concentrations increase the oncotic pressure (colloid-osmotic pressure) of the plasma which is proportional to the number of protein molecules in solution. The influence of serumalbumin is noteworthy since its relatively low molecular weight results in the release of a larger number of molecules. The increase in oncotic pressure favors water retention in the plasma, thus avoiding dehydration during the animals migration to a terrestrial environment.

Several ecologically-based arguments confirm that the adaptation of metamorphosed amphibians to a terrestrial environment is linked to an increase in the concentration of serum proteins, particularly serumalbumin:

—In *Rana* and *Salamandra*, with terrestrial adults, the serum protein concentration increases more rapidly than in *Xenopus* or *Pleurodeles* which can remain perpetually aquatic.

—In *Bufo arenarum*, the serum protein concentration increases faster if the metamorphosed animal migrates to a drier environment.

—In several north-american ambystomatidae, a relationship has been established between the degree of humidity in which the metamorphosed animals live and the concentration in serum proteins [61]. In *Ambystoma macrodactylum* adults, which migrate to a dry environment, these proteins are relatively abundant and suggest an efficient osmotic water retention in the plasma. In *Rhyacotriton olympicus* adults which live in wet areas, proteinemia is lower, resulting in poorer water retention.

2 *Evolution of the hydromineral equilibrium during the transition to a terrestrial environment*

The appearance of new ionic fluxes results in more efficient water conservation when the animals migrate towards a terrestrial environment.

a *Hydromineral equilibrium of premetamorphic larvae.* The aquatic environment of larvae is very diluted compared to their body fluids where sodium (Na^+) and chloride (Cl^-) ions predominate. The loss of these ions by diffusion and the invasion of water by osmosis, through the tegument, are corrected by regulatory mechanisms which allow Na^+ and Cl^- ions to enter and water to be eliminated (Fig. 7A).

Studies carried out in *Rana catesbeiana* tadpoles have shown that the absorption of Na^+ ions occurs through the gill epithelium by an "active transport" mechanism, which requires a source of energy in order for the ions to go against the concentration gradient [20]. Na^+ ions create a higher

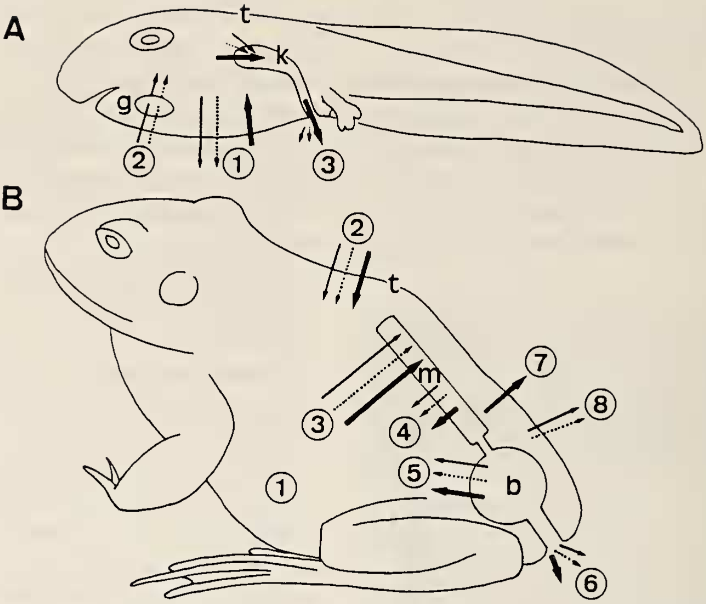

Fig. 7. Changes in the hydric (thick arrows) and ionic (sodium: thin arrows; chloride: dotted arrows) fluxes during anuran metamorphosis. *A*, Premetamorphic tadpole. The effects of the osmotic gain of water and the loss of sodium and chloride ions by diffusion, through the tegument (1), are restricted by branchial ionic absorption (2) and the excretion of an important volume of diluted urine (3). *B*, Adult frog. In a terrestrial habitat, hydric water retention is favoured by a storage of sodium and chloride ions in the body fluids (1, oedema effect). During a stay in a pond, water absorption accompanies the enterance of sodium and chloride ions through the skin (2). Water and ions filtrate rapidly through the glomerulus of the nephrons (3), but are weakly reabsorbed through the wall of the nephric tubules (4). On the contrary, a significant reabsorption occurs through the wall of the urinary bladder (5). Consequently, the urine is slowly excreted (6). The cutaneous loss of water (7) and ions (8) is relatively important. b, urinary bladder; g, gill; k, kidney (pronephros or mesonephros); m, mesonephros; t, tegument. From Hourdry and Beaumont [42].

electric potential on the internal side of the gill epithelium than on the external side, thus allowing Cl^- ions to passively accompany Na^+ ions by simple electrostatic attraction. The gill cells, where "active" Na^+ ion transport takes place, are located at the base of the gills and are equivalent to "chloride cells" in fish. In amphibian larvae, water invasion is also restricted by the excretion of an important volume of diluted urine as in fresh water fish.

b *The appearance of new ionic fluxes during metamorphosis* (Fig. 7B). Metamorphosed amphibians which have left ponds can return to escape from a predator or to reproduce. Na^+ and Cl^- ions transport then takes place towards the body fluids through the epidermis [1, 6, 14, 79]. The amphibians permanent kidney (mesonephros) also recovers Na^+ and Cl^- ions. Part of these ions in solution in the primitive urine crosses the nephron tube wall after being filtered in the glomerulus and returns to the body fluids. The urinary bladder, developed during metamorphosis, represents a third Na^+, Cl^- ion recovery organ [53].

The mechanisms of ion transfer have been defined in the epidermis [14] and in the urinary bladder epithelium [53]. The apical membrane of the epidermal cells becomes permeable to Na^+ ions after acquiring sodium "canals". The "active transport" of these ions towards the body fluids occurs specifically through the basolateral membrane. This "sodium pump" cannot function in lavae because there are no sodium "canals" in which the Na^+ ions can pass. Analogous functional stages can be found in the neoformed urinary bladder epithelium. Cl^- ions cross the intercellular spaces passively, in response to a difference in the trans-epithelial potential due to Na^+ ion movements.

When these different fluxes are taken into account, the concentration of Na^+, Cl^- ions in the body fluids of metamorphosed and terrestrial animals increases in spite of cutaneous losses.

c *Water conservation during the transition to a terrestrial environment.* Water conservation in metamorphosed amphibians migrating to a terrestrial environment is closely linked to the development of new ionic fluxes (Fig. 7B).

The higher quantities of Na^+, Cl^- ions in the metamorphosed animals body fluids favor water conservation in the organism by osmotic retention (oedema effect). The osmotic pressure in the body fluids is lower in winter (300 mosm/l) than in summer (600 mosm/l), when it contributes to slowing down the dehydrating effects of a drier environment.

Each time the animals return to a pond, water along with Na^+, Cl^- ions penetrates by osmosis through the skin into the body fluids.

Even though they were the first organisms to conquer a terrestrial environment, metamorphosed amphibians have retained a fish-type nephron in which a large glomerulus filters a considerable amount of water without much tubular reabsorption. The inadequate adaptation of the kidney to water conservation is compensated by a significant reabsorption of water by the urinary bladder epithelium. Na^+, Cl^- ions are recovered during this reabsorption. Urine excretion is thus slower than in the larva, when taking into account its body weight.

Water retention is also enhanced by several cutaneous modifications linked to metamorphosis, which make the tegument impermeable (superficial keratinisation) or improve its water retaining capacity (mucous film covering the skin, muco-

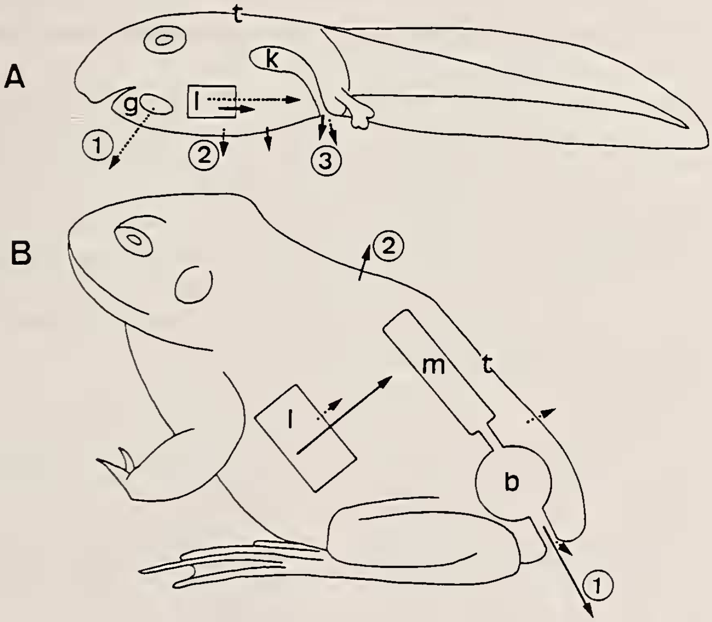

FIG. 8. Shift in the excretion pattern during frog metamorphosis. *A*, Premetamorphic tadpole. The liver (l) preferentially releases ammonia which is mainly excreted as ammonium ions through the gills (1) and in a lesser quantity through the tegument (2) and kidneys (3). *B*, Metamorphosed animal. Urea is synthesized more actively by the liver (l), and then excreted with the urine (1) or through the tegument (2). Dotted arrows: ammonia/ammonium ions fluxes; continuous arrows: urea fluxes; b, urinary bladder; g, gill; k, kidney (pronephros or mesonephros); m, mesonephros; t, tegument. From Hourdry and Beaumont [42].

polysaccharide-rich layer in the dermis).

E TRANSITION TO ANOTHER TYPE OF NITROGEN EXCRETION

Metamorphosed, terrestrial amphibians preserve their hydromineral equilibrium by excreting less urine and developing another type of nitrogen excretion. Most amphibian larvae are ammonotelic, since the ammonia from protein catabolism is essentially excreted as ammonium ions (NH_4^+). Most of these ions are eliminated through the gills while the tegument and the kidneys eliminate the rest (Fig. 8A). In contrast, the juvenile and adult are ureotelic Excremental nitrogen is then preferentially evacuated as urea in solution with the urine or passed through the tegument (Fig. 8B).

The change in the type of nitrogen excretion occurs during metamorphosis. It is associated with more active urea synthesis by the liver using ammonia and carbon dioxide, after a simultaneous and significant increase in the activity of the enzymes controlling the ornithine-urea cycle (carbamyl phosphate synthetase-I, ...) [2, 13].

The transition from ammonotelia to ureotelia in metamorphosed amphibians can be considered as an additional physiological adaptation to a terrestrial environment. Once water conservation mechanisms have been established, the excretion of a weakly toxic waste product such as urea is compatible with the excretion of lower quantities of urine in which this waste product can be concentrated.

After metamorphosis, certain terrestrial anurans (*Phyllomedusa, Chiromantis*) excrete low quantities of urine, particularly rich in uric acid, another weakly toxic waste product [74].

In amphibians such as *Xenopus*, capable of remaining in an aquatic environment after metamorphosis, the invasion of water is limited by the excretion of abundant urine, following low water recovery by the bladder. The NH_4^+ ions are then almost the only urinary nitrogen waste product with a toxicity attenuated by dilution. A tendency toward ureotelia can nevertheless appear in adult *Xenopus* when their environment dries up. Urine excretion then becomes scarce(1)(2).

F SENSORY CHANGES

Sensory organs such as the lateral system are modified during metamorphosis and departure from an aquatic environment [42]. The lateral system is a sensory apparatus which characterizes lower aquatic vertebrates. It is made up of a group of cutaneous receptors (neuromasts). This system could inform the animal of weak water pressure variations and changes in the water currents. It would thus allow the animals to perceive the movement of other organisms at a distance.

In amphibians, the presence of a lateral system is linked to the degree of dependance on the aquatic environment. This system is found in all the larvae. In anurans, it disappears at metamorphosis if the species migrates to a terrestrial environment (*Rana, Bufo, ...*). But it persists if the species remains aquatic (*Xenopus*) [72, 84]. In adults urodeles it does not always completely disappear.

G DEPARTURE FROM WATER: AN ENERGY EXPENDITURE FOR THE ORGANISM

The metamorphic events and departure from water require an energy source (ATP). This energy source is due to the oxydation of substrates such as glucose, fatty acids and glycerol. The glucose comes from the hydrolysis of hepatic glycogen [50, 77]. It is also produced by neoglycogenesis from non-glucidic precursors found in the liver (aminoacids from tissue degeneration,...) [62]. The fatty acids and glycerol come from the hydrolysis of the triglycerides stored in the liver and fatty organ [70].

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(1) In the anura *Scaphiopus couchi*, accidental drainage of their pond is accompanied by an accumulation of urea in the tadpoles body fluids and a transition from ammonotelia to ureotelia. This accumulation of urea leads to osmotic water retention. It enables the tadpoles that have become terrestrial, to wait for a new pond to appear after rain [45].

(2) Certain metamorphosed anurans found in salt marshes in Thailand (*Rana cancrivora*) can withstand osmotic pressures close to those of sea water. The accumulation of urea in their body fluids explains the low water loss observed in these animals [34].

II A MORE RADICAL DEPARTURE FROM WATER CAN ELIMINATE THE AQUATIC LARVAL STAGE

Various amphibians acquire a direct development which puts an end to the free and aquatic larval stage and the need to return to ponds to reproduce [18, 19, 22, 52, 57]. The transition to a direct development has been studied in several oviparous species and also in various other species in which the offspring-parental relationship is more or less pronounced.

A ELIMINATION OF THE AQUATIC LARVAL STAGE IN ANURANS

In certain tree frogs (*Arthroleptis, Chiromantis*) (Fig. 9), the eggs are laid in a substance secreted by the oviducts and beaten into a froth by the legs. This egg cluster hangs over water and when the eggs hatch, the tadpoles fall into the pond where they undergo metamorphosis. In other frogs (Fig. 9), development in an aquatic environment totally disappears. The eggs are laid in a frothy nest (*Leptodactylus fallax*) or in fresh moss (*Thoropa, Leiopelma*) where the larvae hatch and develops. In a Leptodactylidae from the Antilles (*Eleutherodactylus martinicensis*) and in several toads (*Nectophryne*), the eggs are laid on moist, vegetation covered ground and most of the ontogenesis becomes intraovular. The eleutherodactyle embryo has a very vascularized tail fin which is placed against the egg wall and has a respiratory function.

In various anurans, a closer parent-offspring relationship tends to eliminate the free and aquatic larval stage (Fig. 10). This stage is very brief, for example, in a chilian toad (*Rhinoderma darwinii*): the tadpoles that have just hatched in water are swallowed by the males into a vocal sac where they continue their development. After metamorphosis, the juveniles are thrown into the terrestrial environment by a contraction of the sac. In the south-american marsupial tree frog (*Gastrotheca ovifera*), the aquatic development stage completely disappears. The eggs are incubated in the diverticulae of a dorsal skin pouch which develops in reproductive females, under the influence of progesterone secreted by the ovarian follicles after ovulation [16, 17]. Metamorphosis takes place in the skin pouch.

In a guinean anuran, *Nectophrynoides occiden-*

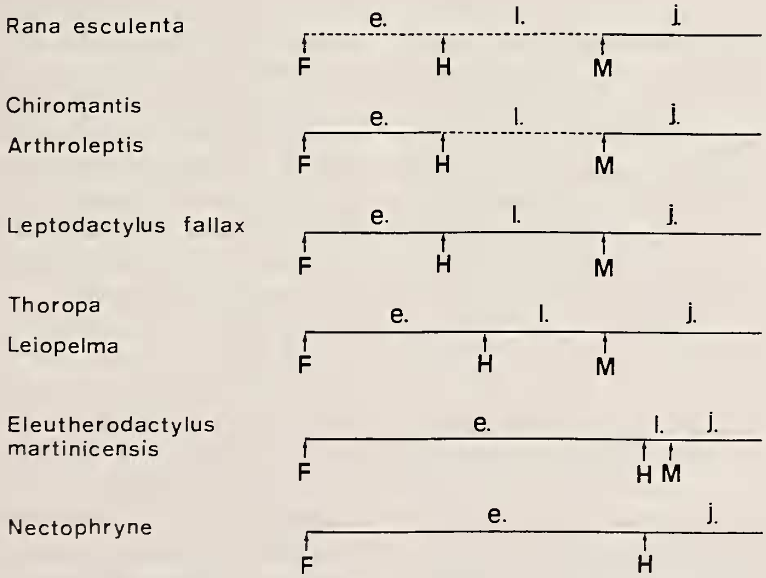

FIG. 9. Transition toward direct development in oviparous anurans. The aquatic larval period is destined to disappear. e, embryo; F, fertilization; H, hatching; j, juvenile; l, larva; M, metamorphosis; continuous line, terrestrial period; dotted line, aquatic period. From Hourdry and Beaumont [42].

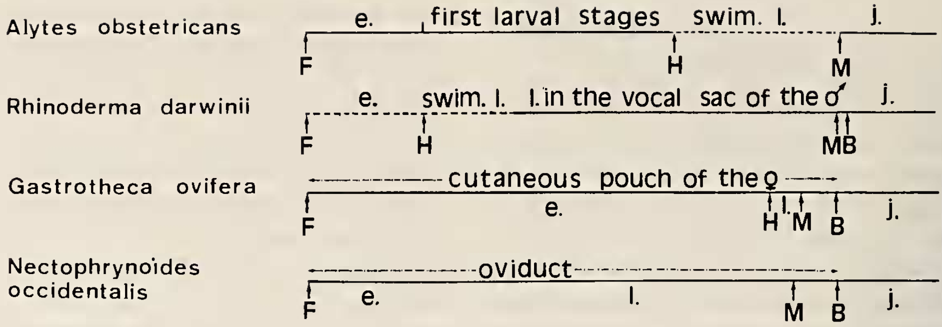

FIG. 10. Transition toward direct development in anurans having different degrees of offspring-parental relationships. B, birth after incubation in a parental cavity (vocal sac, cutaneous pouch, oviduct); e, embryo; F, fertilization; H, hatching; j, juvenile; (swim.) l, (swimming) larva; M, metamorphosis; continuous line, terrestrial period; dotted line, aquatic period. From Hourdry and Beaumont [42].

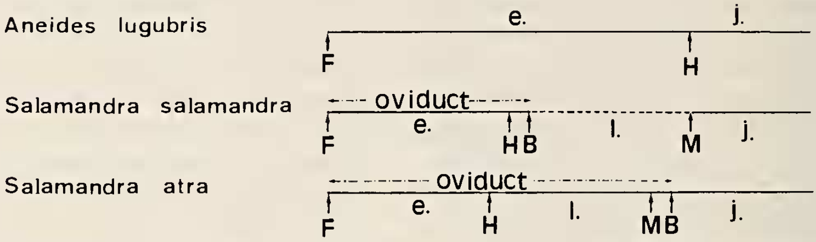

FIG. 11. Development in some urodeles. Abbreviations as in Fig. 10. From Hourdry and Beaumont [42].

talis, the absence of an aquatic stage is due to a particularly close relationship with the mother. Internal fertilization takes place when the cloacae join together. The eggs are incubated in the uterus and produce tadpoles which can ingest the mucoprotein-rich liquid secreted by the uterine wall. This gestation with maternal nutrition (viviparity), however, is not associated with the differentiation of an absorbant placenta. The tadpoles undergo metamorphosis in the genital tract and the female gives birth to adult-like juveniles.

B URODELES (Fig. 11)

American salamanders from the plethodontidae family have a long intra-ovular development and no aquatic stage.

Several european salamanders are viviparous. In the black and yellow salamander (*Salamandra salamandra*), the eggs are incubated in the uterus. The larvae, hatched in the genital tract, are deposited in ponds which they leave after metamorphosis. During their intrauterine period the larvae excrete urea which concentrates in the female genital tract [71]. This waste product is not harmful for the larvae because of its low toxicity. In an aquatic environment, however, these larvae become ammonotelic. The black salamander (*Salamandra atra*) has no aquatic development stage. The larvae hatch and metamorphose into juveniles in the oviducts. After birth these juveniles remain in a terrestrial environment.

C APODES

Apodes (Gymnophiones or Coecilies) are tropical, serpent-like amphibians whose aquatic development stage can also disappear. In the oviparous species *Hypogeophis rostratus* from the Seychelles Islands, a juvenile burrower hatches directly from the egg. In various south-american viviparous species (*Siphonops*), the juveniles emerge from the cloaca and also burrow into the ground.

D CONCLUSION

Direct development without a free aquatic stage can be observed in several amphibian strains. The usual morphological characteristics become indistinct and the various stages "telescope" each other. Metamorphosis, which can nevertheless persist, affects the organs normally used in water but which function here in a terrestrial environment. The tail and the gills, for example, are used by the larvae for aerial breathing.

The hydromineral equilibrium problem is solved by viviparity or by embryonic and larval development in various types of parental pouches. In the oviparous species, embryo dessication is limited by the existence of a thick, impermeable layer of jelly around the eggs.

GENERAL CONCLUSIONS

In amphibians, the departure from water can be considered at two different levels. This emergence occurs at metamorphosis in many species. Nevertheless, certain modifications linked to the phenomenon do not automatically imply such a transition. Tail regression and limb and lung development, on the hand, and the transition from a microphagous to a carnivorous diet, on the other, are not incompatible with remaining in an aquatic environment. The metamorphosed Xenopa for example, swims by rapidly extending its hindlimbs, breathes at the water surface and hunts for food. Other "metamorphic" transformations pre-adapt amphibians better to a terrestrial environment. In *Rana* and *Bufo* adults, this transition is prepared by a more efficient hydromineral equilibrium. In contrast, inefficient water conservation could explain the persistence of an aquatic existence in *Xenopus*, whose hydromineral equilibrium remains that of a fresh water fish.

In fact, metamorphosis does not completely free the adult from an aquatic environment. It can return to avoid predators or to rehydrate its tegument. It must also return to a pond to reproduce. External fertilization and embryo and larva development take place in the pond. In short, the emergence from water is a rather relative notion in certain amphibians. It seems as though their most favorable environment had not yet been decisively determined.

In other amphibians, the departure from an aquatic environment is more radical. It follows a direct development which takes place mostly in the egg, in various types of parental pouches or in the female genital tract. A definate departure from water occurs only in amniote vertebrates. The development of the embryos in an amniotic cavity containing liquid prevents them from dyring out. This type of ontogenesis enabled the amniotes to free themselves entirely from an aquatic environment.

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